Lipids and Signaling on Membrane Surfaces

472-Pos Board B272
Molecular Dynamics Simulations of Monolayers and Membranes with Phosphatidylinositol Bisphosphate
David R. Slochower, Paul A. Janmey.
Proper functionality of biological membranes depends on the regulation of lipid composition and localization. Spatial localization of molecules within the lipid bilayer depends on both steric effects due to their acyl chains and attractive or repulsive interactions between lipid headgroups, such as those mediated by the electrostatic charge of the lipid. Most eukaryotic lipids are zwitterionic or have a charge of −1 at physiological pH, but some lipids such as phosphatidylinositol bisphosphate (PtdInsP_2) bear a net charge of −4. The ability of these highly charged lipids to interact with monovalent and divalent cations affects their spatial organization and temporal distribution on the cytoplasmic side of membranes. In turn, these lipids act as important effectors of apoptosis, inflammation, motility, and proliferation through their interactions with proteins at the membrane interface and transmembrane ion channels. In particular, PtdInsP_2 is able to stabilize the open conformation of both eukaryotic potassium channels and those produced by bacteria, which normally lack PtdInsP_2 lipids in their membrane. We hypothesize that in some settings PtdInsP_2 acts by changing the physical-chemical properties of the membrane rather than by specific biochemical binding to proteins. To explore this hypothesis using molecular dynamics simulations, we completed a set of novel parameterizations at the MP2/6-31+G* level of theory for the three PtdInsP_2 isoforms in several geometries and protonation states. Here we report that there are key differences in the way calcium and magnesium interact with the PtdInsP_2 headgroup and that divalent cations likely influence the propensity of the lipids to form clusters.

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Actin Comets Versus Membrane Ruffles: Distinctive Roles of Phosphoinositides in Actin Reorganization
Tasuku Ueno, Bjoern Falkenburger, Christopher Pohlmeyer,
Takanari Inoue.
Phosphoinositides, a group of lipids found in various cellular membranes, oversee diverse cellular functions including cell proliferation, differentiation, and cell migration. However, the question of how these lipids drive multiple cellular functions and differentially contribute to diseases mediated by aberrant phosphoinositide regulation remains unaddressed.
Using a recently developed technique to rapidly manipulate the level of specific phosphoinositides, we aimed to elucidate a molecular mechanism underlying functional diversity achieved by a limited set of phosphoinositides. In particular, we developed techniques based on rapamycin-induced protein dimerization to rapidly change plasma membrane PIP_2 in order to dissect the role of PIP_2 in actin reorganization. We first increased plasma membrane PIP_2 by rapamycin-induced recruitment of a PIP 5-kinase to the membrane, and found that cells form actin comets. Note that this manipulation increases PIP_2 but depletes PI4P. We then developed a second technique that increases PIP_2 without consuming PI4P, and found - surprisingly - that cells exhibit membrane ruffles. These distinctive phenotypes were critically regulated through two signaling events: 1) a crosstalk between the Rho GTPases Rac and RhoA, and 2) dynamin-mediated vesicular trafficking. We could convert actin comets into ruffles by perturbing these signaling events. As a proof-by-synthesis, we also demonstrated that actin comet formation can be reconstituted without changing the PIP_2 level by combining manipulation of Rho GTPase activity with rapidly induced endocytosis.
In summary, our results indicate that the role of PIP_2 on actin reorganization depends on other phosphoinositides, such as PI4P. This combinatorial regulation may explicate the diversity of phosphoinositide functions.

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A ci-VSP/Tpip Chimera Exhibiting Voltage-Dependent Pi(4,5)P2/PIP3-5'-Phosphatase Activity
Christian R. Halaszovich, Ludivine Frezza, Michael G. Leitner, Daniela N. Schreiber, Carlos A. Villalba-Galea, Dominik Oliver.
Phosphoinositide (PI) concentrations in cell membranes play an important role in many cellular processes. PI phosphatases are crucial for the regulation of these concentrations. Among these phosphatases are the voltage-sensitive phosphatases (VSPs) like the prototypical Ci-VSP, which provide a new paradigm for the control of enzymatic activity. Recently, we showed that voltage sensitivity can be conferred to the cytoplasmic phosphatase PTEN: by fusing the voltage sensor domain (VSD) of Ci-VSP to the catalytic domain (CD) of PTEN, we generated an engineered chimeric VSP, Ci-VSPTEN.
Here we extend this work and report on a chimera created by fusing Ci-VSP's VSD to the CD of the putative PI-phosphatase TPIPα. We demonstrate that this Ci-VSP/TPIP chimera exhibits voltage dependent enzymatic activity. The substrate and position specificity of Ci-VSP/TPIP is analyzed using genetically encoded PI-specific fluorescence labeled probes, the membrane binding of which is assessed using total internal reflection fluorescence (TIRF) microscopy. Control over the membrane voltage is achieved by whole-cell patch clamping.
We find that upon depolarization, the membrane binding of the PIP_3 specific probe, Btk-PH, and the PI(4,5)P_2 specific probe, PLCδ$_1$-PH, decreases, when co-expressed with Ci-VSP/TPIP. In contrast, membrane binding of the PI(3,4)P_2 specific TAPP1-PH and the PI(4)P specific OSBP-PH probes increases upon depolarization.
These findings identify the Ci-VSP/TPIP chimera as a voltage-sensitive PI(4,5)P_2/PIP_3-5'-phosphatase. We conclude that TPIPα is a 5'-phosphatase *in-vivo*, in contrast to PIP_3-3'-phosphatase activity *in-vitro* reported previously (Walker et al., *Biochem. J.* (2001) 360, 277 −283).
These data will help to further the understanding of the mechanism by which voltage control is exerted in VSPs. Additionally, our experiments demonstrate the usefulness of engineered VSPs as a novel tool for the analysis of PI-phosphatases *in-vivo*, an obligatory complement to *in-vitro* characterization.
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Testing the PH Domain Sentry Glutamate Hypothesis: A New Molecular Mechanism of Carcinogenesis
Carissa Pilling, Landgraf Kyle, Falke Joseph.
Several lines of evidence indicate that the E17K glutamate to lysine mutation adjacent to the lipid binding pocket of the PI(3,4,5)P_3 (PIP_3)-specific PH domain AKT1 PH, causes constitutive AKT1 plasma membrane targeting, superactivation and oncogenesis. Recently, we found that the E17K mutant binds the constitutive lipid PI(4,5)P_2 (PIP_2) in addition to the signaling lipid PIP_3, thereby accounting for its constitutive plasma membrane targeting and superactivation (Landgraf, Pilling & Falke (2008) Biochemistry 47:12660-9). The present study tests whether other PIP_3-specific PH domains possess an analogous glutamate residue, which we term the sentry glutamate, adjacent to their PIP_3 headgroup binding pockets. This work has introduced the E345K sentry glutamate mutation into the GRP1 PH domain and tests its effect on domain function. *In vitro* binding studies utilizing synthetic plasma membranes reveal that the E345K sentry mutation dramatically increases PH domain affinity for PIP_2. The resulting PIP_2 affinity is similar to that of the standard PIP_2 sensor PLCδ1 PH. This led us to predict that GRP1 E345K PH would be constitutively targeted to the plasma membrane, like PLCδ1 PH. Live cell experiments reveal that GRP1 E345K PH does indeed constitutively target to the plasma membrane along with PLCδ1 PH in the absence of a PIP_3 signal and this targeting is sensitive to PIP_2 hydrolysis. Thus the E345 glutamate of GRP1 PH is a sentry glutamate that functions to exclude PIP_2 as does the E17 glutamate of AKT1 PH. These findings suggest that sentry glutamate residues are general features of PIP_3-specific PH domains, and that sentry glutamate mutations will be found to underlie multiple human pathologies.

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Assessing the Extent of Membrane Penetration by the Pleckstrin Homology Domain of GRP1: Insights from Molecular Dynamics Simulations
Craig N. Lumb, Phillip J. Stansfeld, Mark S.P. Sansom.
Successful recruitment of peripheral proteins to the cytoplasmic leaflet of the cell membrane is an essential step in several cell signaling pathways, and robust membrane attachment is often achieved via a modular membrane-binding domain. The general receptor for phosphoinositides isoform 1 (GRP1) forms part of the phosphatidylinositol-3-kinase (PI3K) signaling pathway, and contains a pleckstrin homology (PH) domain that allows it to bind reversibly with high specificity to phosphatidylinositol-3,4,5-trisphosphate (PI(3,4,5)P3) in the plasma membrane. GRP1 acts as a nucleotide exchange factor for ADP-ribosylation factor 6 (ARF6), which is involved in membrane trafficking and endocytosis. However, GRP1 is thought to be autoinhibited in the cytosol, only becoming functionally competent when bound to the membrane via its PH domain. While the binding of GRP1-PH to soluble inositol phosphates has been explored in some detail, comparatively little is known about the membrane-associated complex. For example, several membrane-binding modules are able to insert into the membrane but the extent of membrane penetration by GRP1-PH remains unclear. Here, we explore the interaction of GRP1-PH with its target lipid and the surrounding membrane environment using atomistic molecular dynamics simulations. We find that, in addition to the specific interaction with the cognate ligand PI(3,4,5)P3, each of the three flexible loops flanking the binding site experiences nonspecific interactions with the membrane lipids. We investigate how mutation and/or protonation of key amino acid residues on the membrane-binding surface affects membrane penetration, and our results compare favorably with those from experiment. Finally, we employ steered